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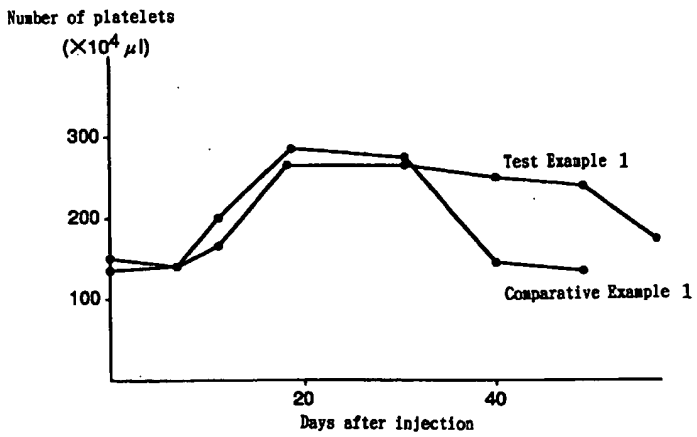
(54) GENE PREPARATIONS

(57) Gene preparations comprising desired genes or vectors containing desired genes integrated thereinto and carriers for supporting the same.

Fig. 1



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Description

FIELD OF THE INVENTION

The present invention relates to the field of medicine, especially gene therapy. Especially, the present invention relates to a preparation containing a gene. The preparation retains a gene vector or a gene stably, releases it gradually and maintains the therapeutic effect for a long time when administered into a living body. The preparation makes it possible to stop a treatment at favorable period.

BACKGROUND OF THE INVENTION

Gene therapy is an active area of research, and expectation for successful therapy is extremely high. In gene therapy approaches in which the gene per se is the medicine, the therapy for disease is conducted by transferring a gene of a designated enzyme, cytokine, and the like into cells of a patient. The introduced gene then produces the designated substance in the patient's body. This gene therapy can be divided to two types. The purpose of the first type is semipermanent expression of designated gene by integrating the gene into genome of a patient. The purpose of another type is transient expression of a gene without expectation of its integration into genome. In the latter type of gene therapy, a method using adenovirus and the like is often adopted as a method for transferring into the patient a gene encoding, for example, a cytokine which increases immuno-potency against cancer cells (Cardiovascular Research, 28, 445 (1994); Science, 256, 808 (1992); Gastroenterology, 106, 1076 (1994); TIBTECH, 11, 182 (1993); J. Biol. Chem., 266, 3361 (1991); Nature Medicine, 1, 583, (1995); and references cited therein).

In the case of using a virus vector, though the efficiency of gene transfer is generally high, repeated administrations are difficult due to immune responses (J. Biol. Chem., 269, 13695 (1994), Am. J. Respir. Cell Mol., 10, 369 (1994)).

A preparation using collagen for gradually releasing medicines containing organic compounds or a protein preparation is disclosed in J. Controlled Release 33, 307-315 (1995), etc. However, the disclosed and usual medicine (for example, protein drug, medicine chemically synthesized, etc.) is dissolved in approximately 1-2 days when retained in, for example, collagen gel.

In a method which is presently used in gene therapy and comprises administering a gene vector (a gene-inserted vector) or a gene directly, the gene vector or the gene contacts cells immediately after administration, and immediately after that gene transfer starts and is completed at once. Furthermore, the gene transduced into cells is diluted (that is, its copy number decreases) with cell division or is reduced by degradation in cells. Therefore, expression of the transferred gene can be maintained only for several weeks which is too short to practice sufficient treatment and this is a deficiency in the method. Accordingly, repeated administrations of a gene vector or a gene are necessary. Therefore, an object of the present invention is to overcome these defects and provide a preparation which can release a gene vector or a gene gradually and can maintain the therapeutic effect for a long time.

Furthermore, a method enabling repeated administrations is expected because those are difficult in gene therapy using a virus vector and the like. Accordingly, a second object of the present invention is to provide a gene preparation enabling repeated administrations in gene therapy using a virus vector and the like.

Furthermore, it is desirable that gene expression in the body can be stopped any time to ensure safety, because a gene is expressed for several weeks which is longer than the term for a protein preparation. Accordingly, a third object of the present invention is to provide a gene preparation which can make it possible to quickly stop the gene transfer when termination of treatment is intended.

SUMMARY OF THE INVENTION

The present inventors have examined various preparations for gradually releasing a gene vector or a gene and have found that, when a preparation wherein a gene or a vector inserted with a gene is incorporated into a carrier made of a biocompatible material was administered into a living body, the gene was unexpectedly expressed for many months. The present inventors have also found that the preparation can be administered repeatedly into a living body and thus the present invention has been accomplished.

Therefore, the characteristic features of the present invention are as follows.

(1) A gene preparation comprising a gene, wherein said gene or a vector inserted with said gene is incorporated into a carrier made of a biocompatible material.

(2) The gene preparation according to (1), wherein said biocompatible material is selected from the group consisting of collagen, gelatin, fibrin, albumin, hyaluronic acid, heparin, chondroitin sulfate, chitin, chitosan, alginic acid, pectin, agarose, hydroxyapatite, polypropylene, polyethylene, polydimethylsiloxane, polymer or copolymer of glycolic acid, lactic acid or amino acid, and a mixture of at least two of these biocompatible materials.

(3) A gene preparation comprising a gene, wherein said gene or a vector inserted with said gene is incorporated into a carrier made of a biocompatible material containing collagen.

(4) A gene preparation comprising a gene, wherein said gene or a vector inserted with said gene is incorporated into a carrier made of collagen.

(5) The gene preparation according to (1), wherein

the content of said biocompatible material in said gene preparation is 0.01-30 w/w% when administered into a living body.

(6) The gene preparation according to (1), which is in a form of a solution, suspension, water-containing gel, film, sponge, rod or sphere.

(7) The gene preparation according to (1), wherein said vector is selected from the group consisting of a virus vector, a liposome vector and a fusogenic liposome vector in which a virus and a liposome are fused.

(8) The gene preparation according to (1), wherein said vector is a virus vector.

(9) The gene preparation according to (1), wherein said vector is an adenovirus vector.

(10) A gene preparation comprising a gene, wherein said gene or a vector inserted with said gene is incorporated into a carrier made of a biocompatible material and said carrier is contained in a vessel through which said gene vector or said gene can penetrate.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 is a graph showing a time course of the number of platelets in Test Example 1 and Comparative Example 1.

Figure 2 is a graph showing a time course of HST-1 in peripheral blood in Test Example 1 and Comparative Example 1.

Figure 3 is a graph showing a time course of the number of platelets in Test Example 4 and Comparative Example 1.

Figure 4 is a graph showing a time course of the number of platelets in Test Example 5.

Figure 5 is a graph showing dose-dependency of collagen on the amount of HST-1 in peripheral blood in Test Example 6.

Figure 6 is a graph showing a time course of the number of platelets in Test Example 7 and Comparative Example 2.

DETAILED DESCRIPTION OF THE INVENTION

The present invention will be described below in more detail.

"A vector for gene insertion" may be any vector that can transfer a gene into cells and includes, for example, a virus vector, a liposome vector, a fusogenic liposome vector in which a virus and a liposome are fused, or the like (Cardiovascular Research, 28, 445 (1994); Science, 256, 808 (1992); Gastroenterology, 106, 1076 (1994); TIBTECH, 11, 182 (1993); J. Biol. Chem., 266, 3361 (1991); Nature Medicine, 1, 583, (1995); and references cited therein).

A virus vector may be any vector that can be used as an ordinary vector in gene therapy and includes, for example, an adenovirus, an adeno-associated virus, a vaccinia virus, a retrovirus, an HIV virus, a herpesvirus, or the like. The gene vector can be obtained by inserting a gene for transfer into a virus vector directly according to a conventional method, for example, described in the above references.

A liposome vector may be any vector that can be used as an ordinary liposome vector in gene therapy and includes, for example, a liposome vector obtained by mixing DOTMA, DOPE, DOGS, etc. When a cationic liposome vector is used, transfer efficiency into cells is high. Examples of the fusogenic liposome vector in which a virus and a liposome are fused include a fusogenic liposome vector in which a Sendai virus (HVJ: hemagglutinating virus of Japan) and a liposome are fused, and the like. A gene vector can be obtained by enclosing a gene for transfer into a liposome vector or a fusogenic liposome vector according to a conventional method, for example, described in the above references. The enclosed gene may be in any form that can express the gene in living body and is preferably a form stable in a living body such as a plasmid, etc.

A gene itself can be retained in a gene preparation of the present invention without being inserted into "a vector for gene insertion" which transfers a gene into cells. In this case, a form of a gene may be any form that can express the gene in living body. For example, a gene may be inserted into what is called an expression vector and the like. A form of a gene is preferably a form stable in a living body such as a plasmid, etc.

"A gene" for transfer may be any gene that can be used in gene therapy and includes, for example, an adenosine deaminase gene, a GM-CSF gene, a thymidine kinase gene, or the like.

"Biocompatible material" is preferably the material which has high biocompatibility and can ratain a gene vector or a gene stably in living body. Examples of the biocompatible material include collagen, gelatin, fibrin, albumin, hyaluronic acid, heparin, chondroitin sulfate, chitin, chitosan, alginic acid, pectin, agarose, hydroxyapatite, polypropylene, polyethylene, polydimethylsiloxane, polymer or copolymer of glycolic acid, lactic acid or amino acid, a mixture of two or more kinds of the biocompatible materials above, and the like. A particularly preferred biocompatible material is collagen. It is also preferred to mix collagen with other biocompatible material described in the above. Collagen may be any collagen and includes, for example, collagen soluble in acid, collagen solubilized by enzyme (for example, atelocollagen, etc.), collagen solubilized by alkali, collagen having modified amino acid side chains, bridged collagen, collagen produced by genetic engineering, or the like. Collagen having modified amino acid side chains includes, for example, succinyl or methyl collagen, or the like. Bridged collagen includes, for example, collagen treated with glutaraldehyde, hexamethylenediisocianate or polyepoxy compound, or the like (Fragrance J., 1989-12, 104-109, Japanese Patent KOKOKU (examined) No.7 (1995)-59522)).

"An additive" may be added to a preparation of the present invention according to necessity in order to stabilize a gene vector and the like, accelerate gene transfer into cells or nuclei or regulate the release of a gene vector and the like. An additive may be any additive that can achieve the purpose and includes, for example, sucrose, glycine, chondroitin sulfate, gelatin, albumin, cytokine, a mixture of the High Mobility Group Proteins HGM-1 and HGM-2 (High Mobility Group-1,-2 Mixture; Experimental Medicine, 12, 184 (1994), BIOTHERAPY, 8, 1265 (1994)), chloroquine, polylysine (Hepatology, 22, 847 (1995)), Transfectam (Trademark, Wako Pure Chemical Industries, Ltd.), or the like.

In the case wherein collagen is mixed with another biocompatible material or an additive, the content of collagen may be at least 10 w/w%, preferably at least 30 w/w% more preferably at least 50 w/w%, and most preferably at least 70 w/w%.

The content of biocompatible material in the gene preparation is varied depending on size, kind and the like of the gene vector or the gene and kind and the like of the biocompatible material.

A preferred content of biocompatible material in a gene preparation is 0.01-30 w/w% under the condition that the gene preparation is in a living body, more preferably 0.05-10 w/w%, and most preferably 0.05-5 w/w%.

Furthermore, the content of biocompatible material in the gene preparation is varied depending on the particular preparation. For example, in the case wherein collagen is used as the biocompatible material, the preferred range of the content will be described below.

When the preparation is in gel form, a preferred content of collagen is 0.01-25 w/w%, more preferably 0.05-10 w/w%, most preferably 0.05-5 w/w%. However, when the content of collagen is 2 % or more, it is preferred that an additive is added at 5-900 w/w% of collagen.

When the preparation is in film form, a preferred content of collagen is 0.2-30 w/w% as a content before drying, more preferably 0.3-5 w/w%, most preferably 0.4-2 w/w%. However, when a content of collagen is 1 % or more, it is preferred that an additive is added at 5-900 w/w% of collagen.

When the preparation is in a solid rod form, the preferred content of collagen is 10-30 w/w% and it is preferred that an additive is added at 5-100 w/w% of collagen.

A gene preparation of the present invention is not limited to a specific form. The preparation can be in solution, suspension, water-containing gel, film or sponge. Solid forms can be shaped as a rod, sphere and the like. However, a preferred form is generally a solution, suspension or water-containing gel, though the form of the preparation is varied depending on kind, size and the like of a vector for gene insertion.

A gene preparation is obtained by keeping a content of biocompatible material in the gene preparation within the above preferred range under the condition that the gene preparation is in a living body.

Examples of methods for producing a gene preparation in a form of solution, suspension or water-containing gel include (1) a method which comprises mixing a powder, solution, suspension or gel of a gene vector or a gene (referred to as a gene vector and the like below) with a carrier in a form of a solution or gel to which an additive is added according to necessity, (2) a method which comprises making a solution, suspension or gel of a gene vector and the like penetrate into a carrier that is in powder form to which an additive is added according to necessity, or (3) a method which comprises making a solution, suspension or gel of a gene vector and the like penetrate into a carrier that is a sponge to which an additive is added according to necessity and kneading them together. The methods for making a gene preparation of the present invention are not limited to such methods.

Examples of methods for producing a gene preparation in a solid form include (1) a method which comprises mixing a powder, solution, suspension or gel of a gene vector and the like with a carrier in a form of solution or gel to which an additive is added according to necessity and drying the mixture, (2) a method which comprises mixing a powder, solution, suspension or gel of a gene vector and the like with a carrier in powder form to which an additive is added according to necessity and drying the mixture, (3) a method which comprises making a solution, suspension or gel of a gene vector and the like penetrate into a carrier in the form of a sponge to which an additive is added according to necessity and drying the sponge, (4) a method which comprises making a solution, suspension or gel of a gene vector and the like penetrate into a carrier in the form of a sponge to which an additive is added according to necessity and drying the sponge as it stands or kneading and drying the sponge after adding water and the like according to necessity, (5) a method which comprises crushing and press-molding a solid product obtained by a method according to (1) to (4), or (6) a method which comprises mixing a powder of a gene vector and the like with a carrier that is a powder to which an additive is added according to necessity and press-molding the mixture. The present invention is not limited to such methods. The drying method; temperature and moisture at drying; mixing method; temperature and moisture at mixing; method for press-molding; temperature, moisture and compressing pressure at press-molding; solution velocity of carrier solution and gene vector solution; and solution velocity of mixture of carrier and gene vector solution; and pH may be as same as those in conventional methods.

A gene preparation of the present invention can be administered by various methods according to the disease being treated, the targeted organ and the like. For example, a gene preparation of the present invention can be administered subcutaneouly or intramuscularly,

or can be administered directly to targeted sites of disease such as kidney, liver, lung, brain and the like. Direct administration to disease site enables organ-selective therapy.

According to the present invention, when a biodegradable material is used as the biocompatible material, it is not necessary to take the biocompatible material out of the body after administration. Furthermore, repeated administrations are possible.

On the other hand, when discontinuance of gene transfer is required depending on the kind or condition of a disease, a gene preparation can be taken out as it stands, and gene transfer can be stopped. For example, if a gene preparation is a solid, it can be taken away by surgery or the like. When gene therapy is conducted by using a gene preparation wherein a gene vector and the like is retained in a vessel or the like having pores through which a virus can pass freely, the vessel or the like can be taken away at termination of the treatment. For example, gene therapy can be conducted by using a vessel (tube) as described in Japanese patent application No. 3(1991)-120115 (International publication number WO92/20400).

A gene preparation of the present invention can gradually release a gene vector and the like and simultaneously can retain a gene-inserted vector and the like stably in a living body during the sustained release. Therefore, the time of effective gene expression after a single administration can be extended by controlling the period of gene transfer into cells by delaying contact between cells and vectors.

A gene preparation of the present invention also enables repeated administrations of a gene vector such as a virus vector and the like which is otherwise difficult to administer repeatedly due to appearance of neutralizing antibodies in a subject.

Furthermore, a gene preparation of the present invention enables regulation of gene expression because the gene preparation can be taken away when termination of treatment is intended.

Examples

Example 1

A gene preparation was prepared by mixing 0.9 ml of culture medium containing 10^9 pfu (plaque forming unit) of an adenovirus (Adex1 HST-1; Proc. Natl. Acad. Sci. USA, Vol. 91, 12368 (1994)) inserted with a gene encoding fibroblast cell growth factor HST-1 (FGF4) (prepared according to the method described in Proc. Natl. Acad. Sci. USA, 84, 2980-2984 (1987)) with 0.1 ml of neutral solution of atelocollagen (atelocollagen implant produced by KOKEN: 2% atelocollagen solution). The above adenovirus (Adex1 HST-1) can be obtained by deleting part of E1A, E1B and E3 of adenovirus type 5 (ATCC catalog No. VR-5) according to a method described in J. Virol., 54, 711-719 (1985) or Proc. Natl. Acad. Sci. USA, 93, 1320 (1996) and Cell, 13, 181-188 (1978); inserting a gene of fibroblast cell growth factor HST-1 into the non-proliferative-type adenovirus gene.

Example 2

A gene preparation in gel form is prepared by mixing 0.1 ml of 2% atelocollagen neutral solution with 0.9 ml of culture medium containing 10^9 pfu of Adex1 HST-1 and then keeping the mixture at 37 °C.

Example 3

A gene preparation is prepared by obtaining a sponge by lyophilizing atelocollagen neutral solution; cutting the sponge about 5 mm of square; adding the cut sponge to 1 ml of culture medium containing 10^9 pfu of Adex1 HST-1; and letting the sponge stand overnight.

Example 4

A gene preparation is prepared by lyophilizing a gene preparation in gel form that is prepared in Example 2

Example 5

A gene preparation in pellet form (a compressed product in the shape of a rod) is prepared by again lyophilizing the gene preparation that is obtained in Example 3 and compressing the lyophilized sponge into a rod shape.

Example 6

A gene preparation is prepared by mixing a plasmid vector which is obtained by inserting fibroblast cell growth factor HST-1 (FGF4) into an expression vector (pRc/CMV) having a cytomegalovirus (CMV) promoter with a liposome (DMRIE-C (produced by GIBCO-BRL)) and then mixing a solution containing the liposome with the same volume of atelocollagen neutral solution.

Example 7

A gene preparation in bead form is prepared by mixing 1 ml of culture medium containing 10^9 pfu of Adex1 HST-1 with 1 ml of 1% alginic acid solution; adding the alginic acid solution containing the vector drop by drop to 0.5% calcium chloride solution through a nozzle to obtain beads of 1 mm diameter; and collecting the beads by centrifugation.

Example 8

A gene preparation in bead form is prepared by mixing 1 ml of culture medium which contains 10^9 pfu of

Adex1 HST-1 with 1 ml of 5% agarose gel solution warmed to 45 °C; adding the mixed solution drop by drop to a phosphate buffered saline solution at 10 °C through a nozzle to obtain beads of 1 mm diameter; and collecting the beads by centrifugation.

Example 9

A gene preparation is prepared by enclosing the gene preparation which was obtained in Example 1 in a bag made of polyester (made from artificial blood vessel) (Micron (trademark, produced by INTERVASCULAR).

Example 10

As same as Example 1, 1 ml of a gene preparation was prepared by mixing culture medium containing 10^9 pfu of Adex1 HST-1 with atelocollagen neutral solution so as to come to be 0.1, 0.2, 0.4, 1.0 or 2.0 w/w% in final concentration of atelocollagen.

Example 11

A gene preparation in gel form is prepared by mixing 5 ml of aqueous solution which contains a plasmid pOG44 (purchased from Stratagene) at a concentration of 73.25 μg/ml with 5 g of atelocollagen acid solution (containing 2 % atelocollagen, pH 3.5); lyophilizing the mixture to obtain a sponge; adding distilled water to the sponge so as to come be 0.4, 2, 5, 10 or 20 w/w% in a concentration of atelocollagen.

Example 12

A gene preparation in gel form is prepared by mixing 5 ml of aqueous solution which contains a plasmid pOG44 at a concentration of 73.25 μg/ml with 5 g or 2.5 g of atelocollagen acid solution; adding 260 μl or 640 μl of aqueous solution of human serum albumin (80 mg/ml) to the mixture and mixing them; lyophilizing the mixture to obtain a sponge; adding distilled water to the sponge so as to come be 10 w/w% in the total concentration of atelocollagen and human serum albumin.

Example 13

A gene preparation in a form of film is prepared by spreading and gradually drying the gene preparation in gel form that is obtained in Example 11 on a glass.

Example 14

A gene preparation in rod form is prepared by squeeze-molding and gradually drying the gene preparation in gel form that is obtained in Example 11 or 12.

Comparative Example 1

One ml of culture medium containing 10^9 pfu of Adex HST-1 was administered intraperitoneally to a mouse. Then, on about the 12th day after administration, the number of platelets increased approximately two-fold, and this effect lasted to the 20-30th day. Furthermore, the concentration of HST-1 in peripheral blood was 50 ng/ml and at its maximum on the 20th day after administration. However, the concentration of HST-1 could not be maintained at a fixed level differently from the preparation of Example 1 below and HST-1 could not be detected in blood on the 60th day after administration. These results are shown in Figures 1 and 2.

Test Example 1

1.0 ml of a gene preparation which had been prepared in Example 1 was administered intraperitoneally to a mouse. Then, on about the 12th day after administration, the number of platelets increased approximately two-fold, and this effect lasted beyond the 50th day. Furthermore, the concentration of HST-1 in peripheral blood was maintained at 20 ng/ml beyond the 80th day after administration. These results are shown in Figures 1 and 2.

Test Example 2

1.0 ml of a gene preparation prepared as in Example 2 is administered intraperitoneally to a mouse. Then, on about the 12th day after administration, the number of platelets increases approximately two-fold, and this effect lasts beyond the 60th day after adminiatration.

Test Example 3

A gene preparation prepared as in Example 3 is administered intraperitoneally to a mouse. Then, on about the 12th day after administration, the number of platelets increases approximately two-fold, and this effect lasts beyond the 60th day after administration.

Test Example 4

A gene preparation which had been prepared in Example 1 was administered intraperitoneally to a mouse, and the gene preparation was taken out on the third day after administration. As a result, on about the 12th day after administration the number of platelets approximately doubled, then decreased and returned to the normal level on about the 25th day after administration. These results are shown in Figure 3.

Test Example 5

A gene preparation which had been prepared in Example 1 was administered intraperitoneally to a

mouse and the gene preparation was taken out on the third day after administration. On the 20th day after administration a gene preparation which had been prepared in Example 1 was administered again. As a result, on about the 12th day after administration the number of platelets approximately doubled, then decreased and returned to the normal level on about the 20th day after administration. Immediately after the second administration, the number of platelets again increased approximately two-fold, and this effect lasted beyond the 40th day after the first administration. These results are shown in Figure 4.

Test Example 6

Each of 5 gene preparations which had been prepared in Example 10 or 1 ml of culture medium which contained 10^9 pfu of Adex HST-1 and was completely free from collagen was administered intraperitoneally to a mouse. The concentration of HST-1 protein in peripheral blood was determined on the 5th day after administration. The results are shown in Figure 5. The data show that the serum concentration of HST-1 protein deceased with increasing concentration of collagen in the preparation.

Test Example 7

As a result that 1 ml of culture medium containing 10^9 pfu of Adex HST-1 was administered intraperitoneally to a mouse, the number of platelets approximately doubled by the 12th day after administration, then decreased and returned to the normal level on about the 35th day after administration. On the 37th day after the first administration, 1 ml of an atelocollagen-containing gene preparation which had been prepared in Example 1 was administered again. Immediately after that, the number of platelets increased approximately two-fold, and this effect lasted throughout the remaining term of the experiment. These results are shown in Figure 6.

Comparative Example 2

One ml of culture medium containing 10^9 pfu of Adex HST-1 was administered intraperitoneally to a mouse and again administered on the 37th day after the first administration. As a result, on about the 12th day after the first administration the number of platelets approximately doubled, then decreased and returned to the normal level on about the 35th day after the first administration. Platelets did not increase after the second administration of Adex HST-1. These results are shown in Figure 6.

The results in Test Example 7 and Comparative Example 2 reveal that it is possible to administer a gene preparation prepared in Example 1 repeatedly in contrast with the result obtained upon repeated administrations of an adenovirus vector not formulated according to the present invention. The inability to make a repeated administration is due to the appearance of neutralizing antibodies.

Claims

1. A gene preparation comprising an intended gene, wherein said gene or a vector inserted with said gene is incorporated into a carrier made of a biocompatible material.

2. The gene preparation according to Claim 1, wherein said biocompatible material is selected from the group consisting of collagen, gelatin, fibrin, albumin, hyaluronic acid, heparin, chondroitin sulfate, chitin, chitosan, alginic acid, pectin, agarose, hydroxyapatite, polypropylene, polyethylene, polydimethylsiloxane, polymer or copolymer of glycolic acid, lactic acid or amino acid, and a mixture of at least two of these biocompatible materials.

3. A gene preparation comprising an intended gene, wherein said gene or a vector inserted with said gene is incorporated into a carrier made of a biocompatible material containing collagen.

4. A gene preparation comprising an intended gene, wherein said gene or a vector inserted with said gene is incorporated into a carrier made of collagen.

5. The gene preparation according to Claim 1, wherein the content of said biocompatible material in said gene preparation is 0.01-25 w/w% when administered into a living body.

6. The gene preparation according to Claim 1, which is in a form of a solution, suspension, water-containing gel, film, sponge, rod or sphere.

7. The gene preparation according to Claim 1, wherein said vector is selected from the group consisting of a virus vector, a liposome vector and a fusogenic liposome vector in which a virus and a liposome are fused.

8. The gene preparation according to Claim 1, wherein said vector is a virus vector.

9. The gene preparation according to Claim 1, wherein said vector is an adenovirus vector.

10. A gene preparation comprising an intended gene, wherein said gene or a vector inserted with said gene is incorporated into a carrier made of a biocompatible material and said carrier is contained in a vessel through which said gene vector or said

gene can penetrate.

11. A method for treating a living body with a gene, which comprises the step of administering a gene preparation comprising an intended gene, wherein said gene or a vector inserted with said gene is incorporated into a carrier made of a biocompatible material.

Fig. 1

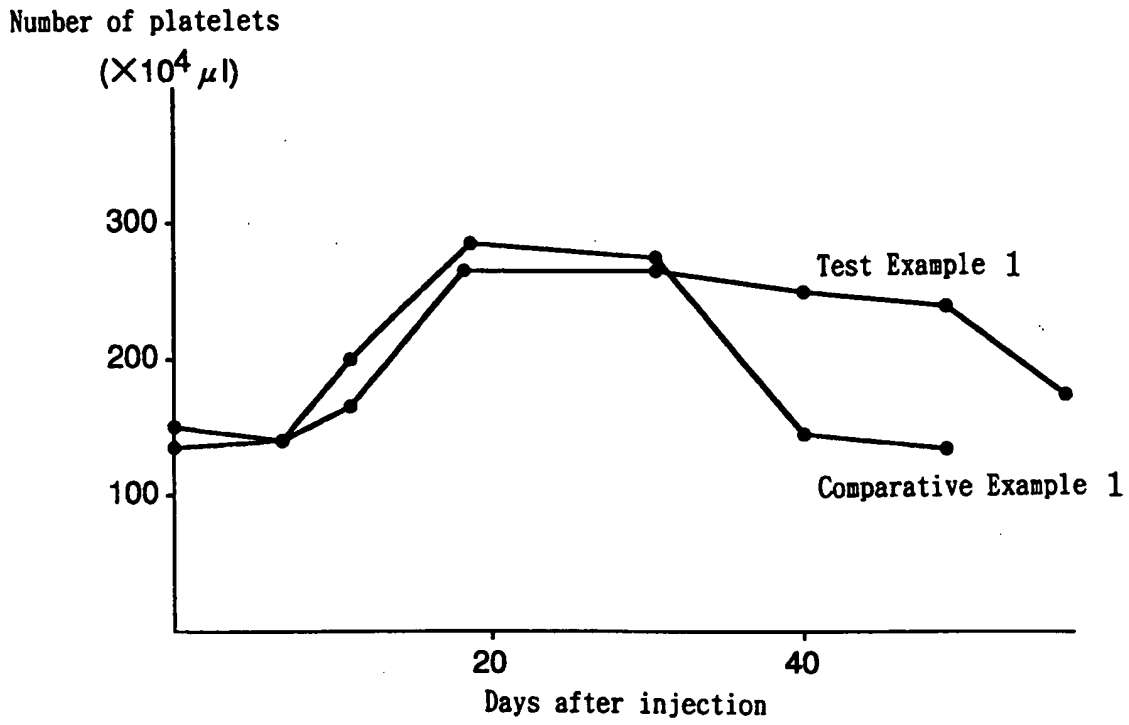

Number of platelets
(×10⁴ μl)
300
200
100
Test Example 1
Comparative Example 1
20
40
Days after injection

Fig. 2

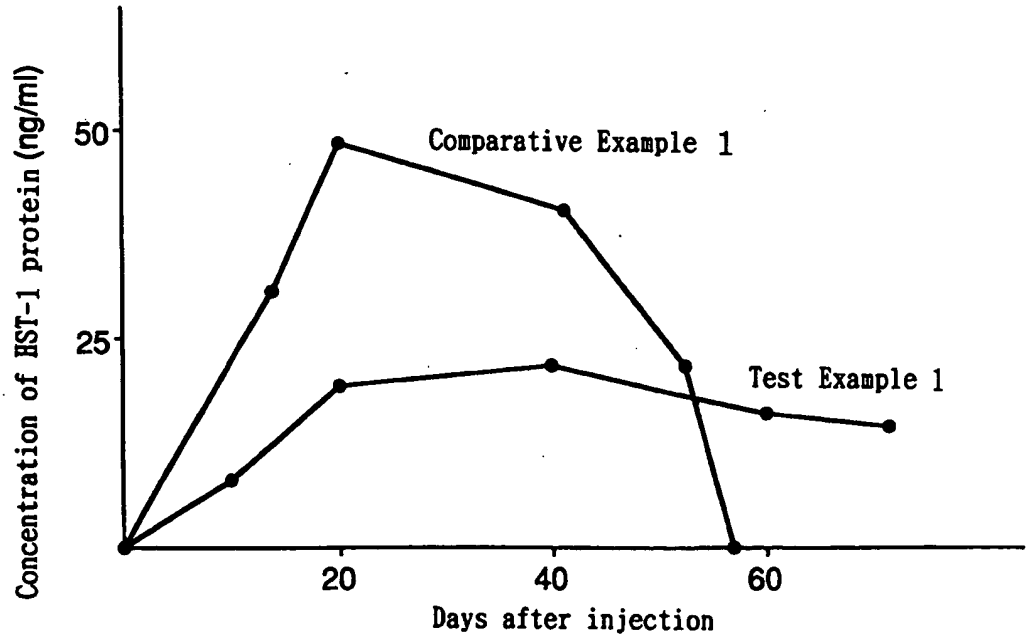

Concentration of HST-1 protein (ng/ml)
50
25
Comparative Example 1
Test Example 1
20
40
60
Days after injection

Fig. 3

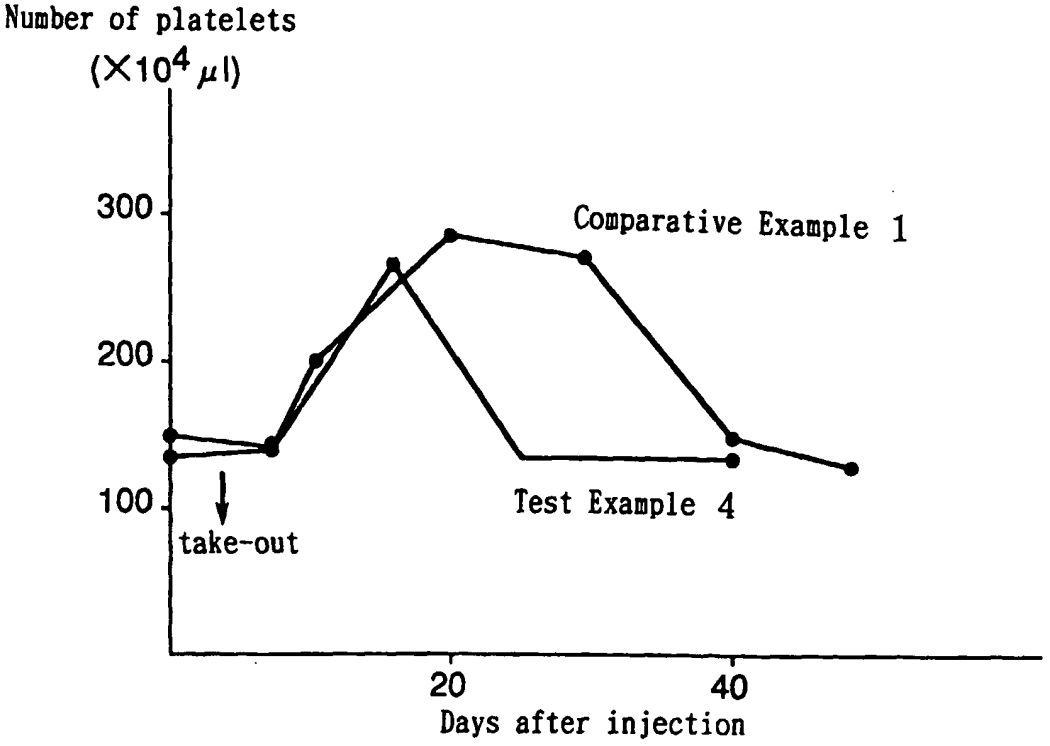

Number of platelets
(×10⁴ μl)
300
200
100
Comparative Example 1
Test Example 4
take-out
20
40
Days after injection

Fig. 4

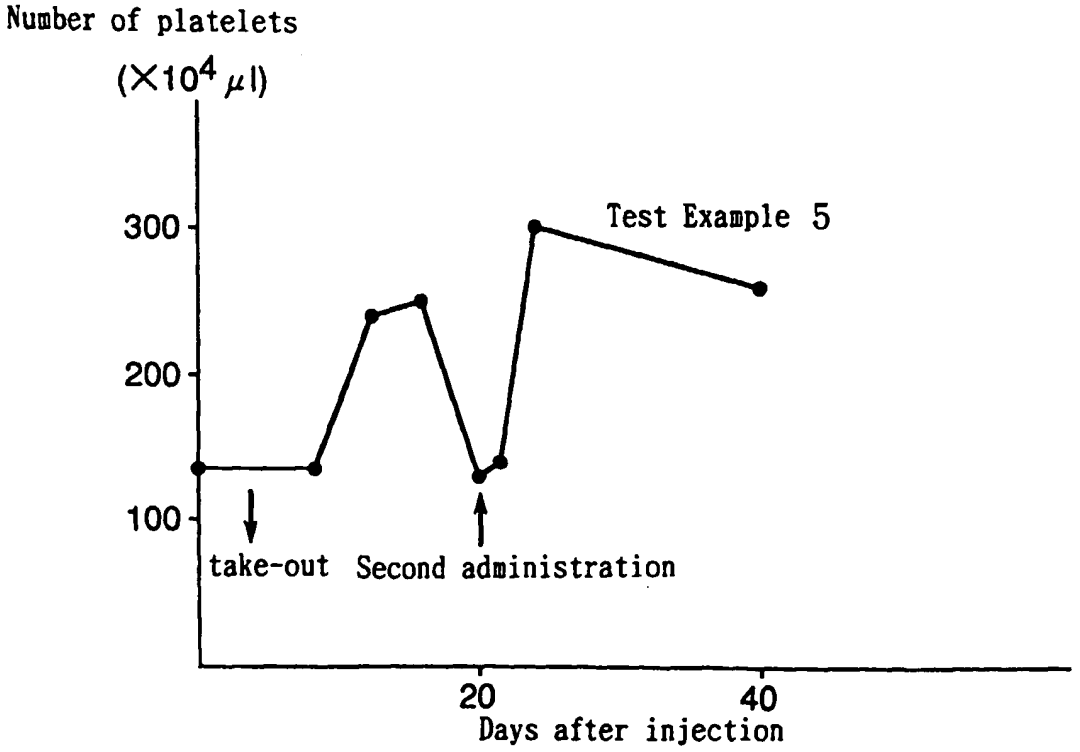

Number of platelets
(×10⁴ μl)
300
200
100
Test Example 5
take-out
Second administration
20
40
Days after injection

Fig. 5

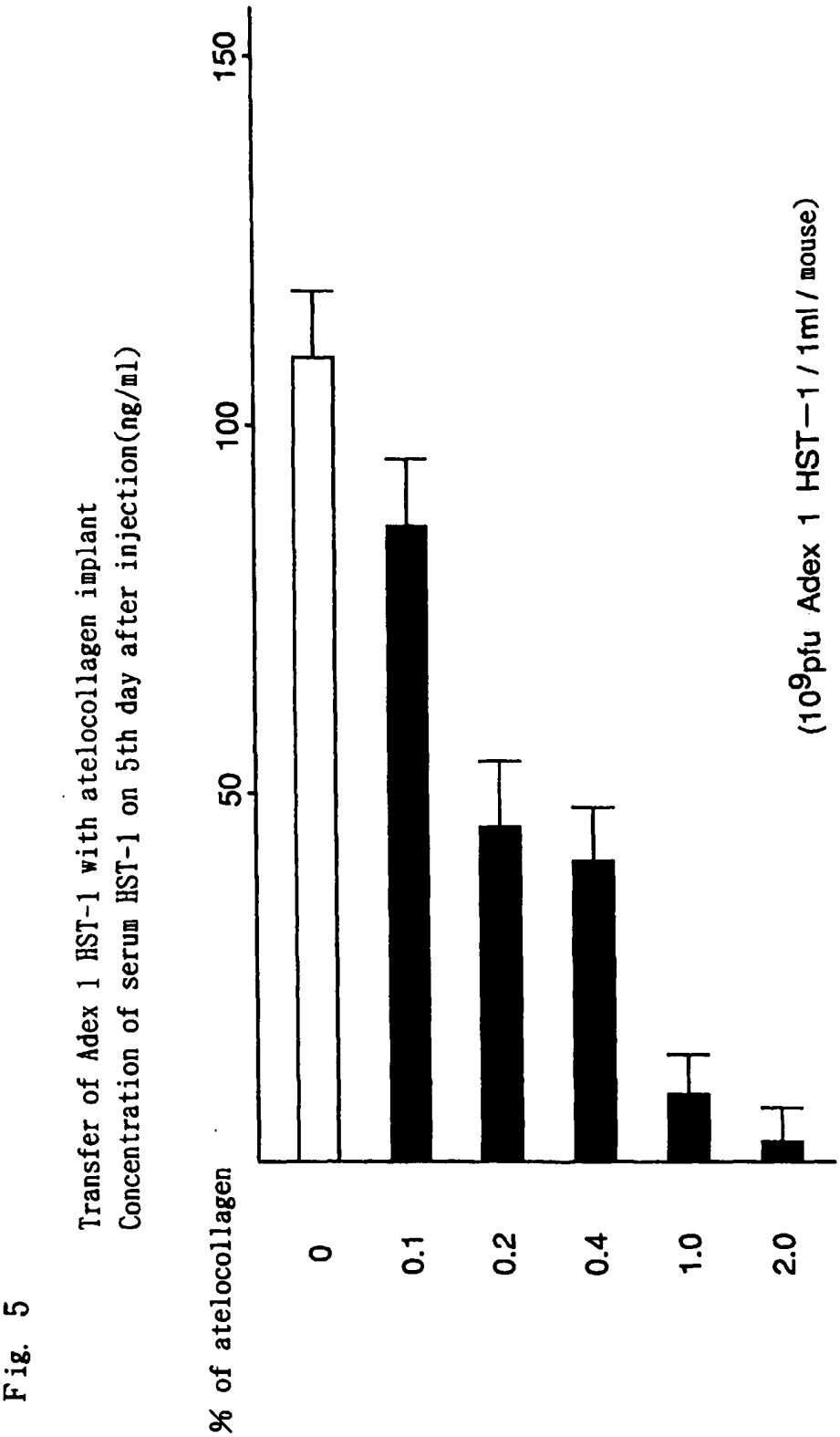
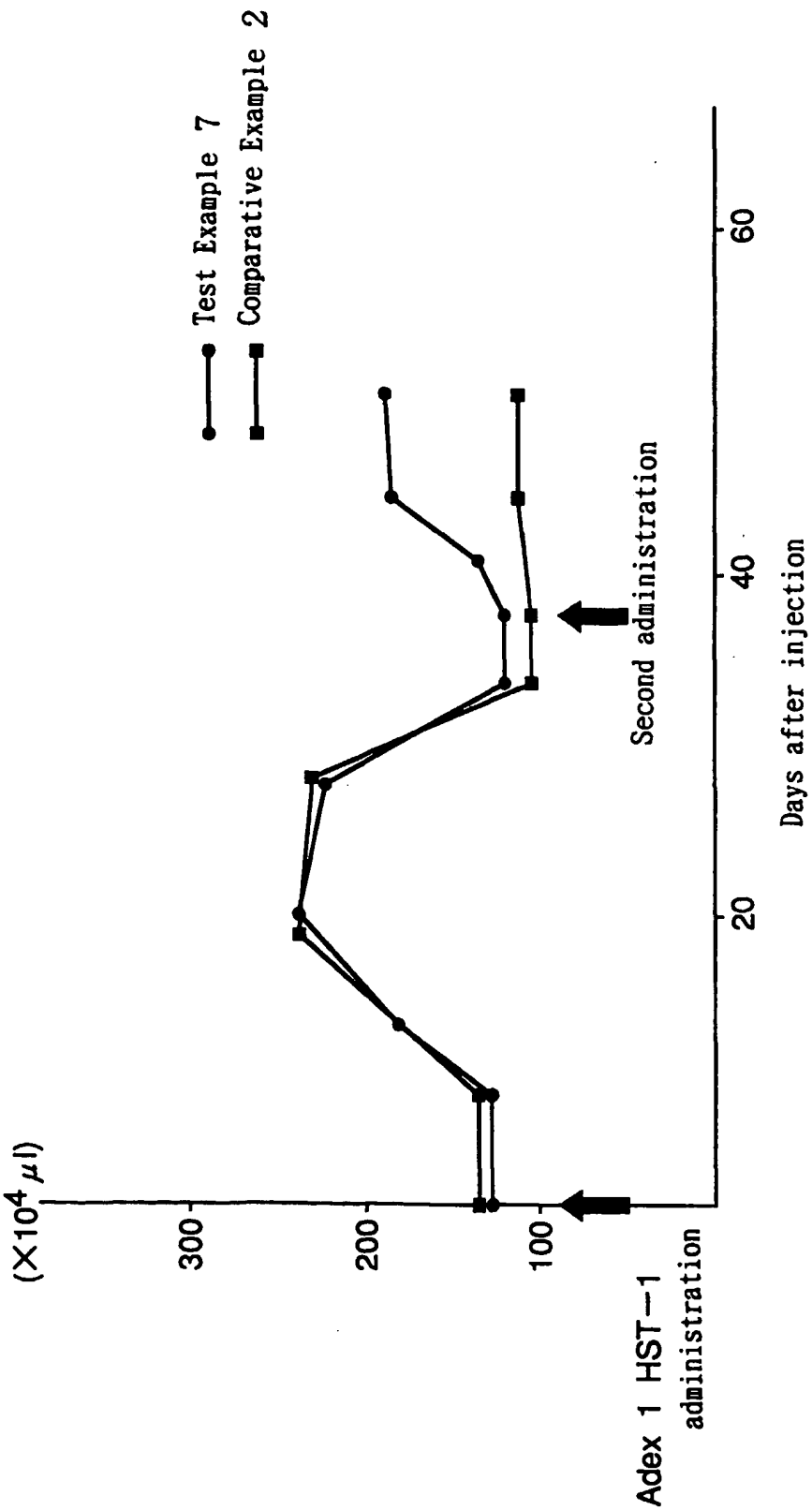

Transfer of Adex 1 HST-1 with atelocollagen implant
Concentration of serum HST-1 on 5th day after injection(ng/ml)
150
100
50
% of atelocollagen
0
0.1
0.2
0.4
1.0
2.0
(10^9pfu Adex 1 HST−1 / 1ml / mouse)

Fig. 6 Repeated administrations of Adex 1 HST-1 with atelocollagen implant



INTERNATIONAL SEARCH REPORT

International application No.
PCT/JP96/01824

A. CLASSIFICATION OF SUBJECT MATTER
Int. Cl^6 A61K48/00, 9/127, 31/70, 35/76, 47/02, 47/32, 47/34, 47/36, 47/42
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED
Minimum documentation searched (classification system followed by classification symbols)
Int. Cl^6 A61K48/00, 9/127, 31/70, 35/76, 47/02, 47/32, 47/34, 47/36, 47/42

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	JP, 62-228028, A (Sumitomo Pharmaceuticals Co., Ltd. and another), October 6, 1987 (06. 10. 87) & EP, 230647, A & US, 4849141, A	1 - 10
Y	JP, 3-163032, A (Sumitomo Pharmaceuticals Co., Ltd.), July 15, 1991 (15. 07. 91) & EP, 412554, A	1 - 10
Y	JP, 3-93716, A (Koken K.K.), April 18, 1991 (18. 04. 91)(Family: none)	1 - 10
Y	JP, 61-236729, A (Sumitomo Pharmaceuticals Co., Ltd.), October 22, 1986 (22. 10. 86) & US, 4774091, A	1 - 10
Y	JP, 60-126217, A (Sumitomo Chemical Co., Ltd.), July 5, 1985 (05. 07. 85) & EP, 139286, A	1 - 10

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier document but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
September 19, 1996 (19. 09. 96)	October 1, 1996 (01. 10. 96)
Name and mailing address of the ISA/ Japanese Patent Office Facsimile No.	Authorized officer Telephone No.

Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/JP96/01824

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	WO, 92/20400, A (Sumitomo Pharmaceuticals Co., Ltd. and another), November 26, 1992 (26. 11. 92) & JP, 4-509656, A & EP, 586700, A	1 - 10
Y	JP, 1-68278, A (Hoechst AG.), March 14, 1989 (14. 03. 89) & EP, 304700, A & US, 4941874, A	1 - 10

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/JP96/01824

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.: 11
because they relate to subject matter not required to be searched by this Authority, namely:
Claim 11 pertains to methods for treatment of the human or animal body by therapy, and thus relates to a subject matter which this International Searching Authority is not required, under the provisions of Article 17(2)(a)(i) of the PCT and Rule 39.1(iv) of the Regulations under the PCT, to search.

2. [ ] Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

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